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IN RE APPLICATION OF :

Mitsuko ISHIHARA et al : EXAMINER: CHAKRABARTI, A.

SERIAL NO: 09/892,485 :

FILED: JUNE 28, 2001 : GROUP ART UNIT: 1634

FOR: METHOD FOR DETECTING ENDOCRINE DISRUPTING ACTION OF A TEST SUBSTANCE :

"RESPONSE UNDER 37 CFR 1.116– EXPEDITED PROCEDURE EXAMINING GROUP 1634 "

Do not Enter
/A. Chakrabarti/
11/5/02

AMENDMENT & REQUEST FOR RECONSIDERATION

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

In response to the Official Action, mailed August 27, 2002, the Applicants respectfully request reconsideration of the rejections of record in view of the following amendments and remarks.

IN THE CLAIMS

Cancel Claims 1-28.

Add new Claims 29-65.

--29. (New) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing a cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell that is sensitive to an endocrine hormone in the presence of the

endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or

(C) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or

(D) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression pattern (4) of said cell; and

(E) comparing gene expression pattern (1) with gene expression pattern (2), (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2), (3) and/or (4) is indicative of endocrine disrupting action by the test substance.

30. (New) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2).

31. (New) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (3).

32. (New) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2) and gene expression pattern (3).

33. (New) The method of Claim 29, comprising comparing gene expression pattern (1) with gene expression pattern (4).

34. (New) The method of Claim 29, wherein said gene expression patterns are measured by determining the variation in the amount of gene transcription.

35. (New) The method of Claim 29, comprising recovering RNA corresponding to

each gene expression pattern, optionally producing cDNA corresponding to said RNA, and comparing said RNA or cDNA of (A) with that of (B), (C) and/or (D) to determine the endocrine disrupting activity of the test substance, wherein a difference in the amount of RNA or cDNA between (A) and (B), (C) and/or (D) is indicative of the endocrine disrupting activity of the test substance.

36. (New) The method of Claim 29, wherein the RNA, or optionally the cDNA corresponding to said RNA, obtained from (A) and (B), (C) and/or (D) is electrophoretically separated to determine the gene expression patterns.

37. (New) The method of Claim 35, wherein the RNA, or optionally the cDNA corresponding to said RNA, obtained from (A) is hybridized to the RNA or cDNA obtained from (B), (C) or (D), and the gene patterns of (A) and (B), (C) or (D) are determined after subtraction of the hybridizing RNA or cDNA.

38. (New) The method of Claim 29, wherein endocrine disruption is determined by identifying one or more types of RNA expressed in (A), but not expressed in (B), (C) or (D), or alternatively, one or more types of RNA expressed in (B), (C) or (D), that are not expressed in (A).

39. (New) The method of Claim 29, wherein endocrine disruption is determined by identifying that a different amount of one or more types of RNA is expressed in (A), compared to (B), (C) or (D).

40. (New) The method of Claim 29, comprising:

(a) recovering RNAs from (A) and (B), (C) and/or (D);

(b) subjecting the RNAs recovered in step (a) to reverse transcription;

(c) amplifying reverse transcription products obtained in (b) by PCR; and

(d) subjecting PCR products obtained in step (c) to electrophoresis, comparing

the electrophoretic patterns of bands obtained, thereby detecting a band specific to a first gene expression pattern of (A).

41. (New) The method of Claim 29, wherein said gene expression patterns are measured by determining variation in the amount of protein or glycoprotein expression between (A) and (B), (C) and/or (D).

42. (New) The method of Claim 29, wherein one or more protein(s) or glycoprotein(s) expressed in (A) and (B), (C) and/or (D) are electrophoretically separated to determine the gene expression patterns.

43. (New) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B), (C) and/or (D) are electrophoretically separated using SDS-PAGE to determine the respective gene expression patterns.

44. (New) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B), (C) and/or (D) are electrophoretically separated using two-dimensional electrophoresis to determine the respective gene expression patterns.

45. (New) The method of Claim 29, wherein endocrine disruption is determined by identifying that a different amount of a protein or glycoprotein is expressed in the gene expression pattern of (A), compared to (B), (C) or (D).

46. (New) The method of Claim 29, comprising determining a variation in the amount of protein modification in said gene expression patterns, wherein a variation in protein modification between one or more proteins in the gene expression pattern of (A) compared to (B), (C) and/or (D) is indicative of an endocrine disrupting activity of the test substance.

47. (New) The method of Claim 46, where the variation in protein modification is measured by:

recovering the glycosylated proteins of (A), and (B), (C) and/or (D) by binding them to a substance that binds to a polysaccharide chain,

cleaving the polysaccharide chain from the glycoprotein, and

determining the gene expression patterns obtained from (A) and (B), (C) and/or (D) based on a comparison of the glycoproteins after cleavage.

48. (New) The method of Claim 29, wherein said cell is a germ cell.

49. (New) The method of Claim 29, wherein said cell is a nerve cell.

50. (New) The method of Claim 29, wherein said cell is a normal cell.

51. (New) The method of Claim 29, wherein said cell is a cancer cell.

52. (New) The method of Claim 29, wherein said cell is a nonhuman mammalian cell.

53. (New) The method of Claim 29, wherein said cell is a human cell.

54. (New) The method of Claim 29, wherein said cell is not a genetically engineered cell.

55. (New) The method of Claim 29, wherein said cell is selected from the group consisting of a murine neuroblastoma cell, a murine uterus carcinoma cell, a murine testicular Leydig cell, a cell derived from testicular Sertoli cells.

56. (New) The method of Claim 29, wherein said cell is selected from the group consisting of Neuro2a, MCF7, TM3, TM4, 15P-1 and S-20Y.

57. (New) The method of Claim 29, wherein said endocrine hormone is a female hormone.

58. (New) The method of Claim 29, wherein said endocrine hormone is estrogen, estradiol, or progesterone.

59. (New) The method of Claim 29, wherein said endocrine hormone is a male

hormone.

60. (New) The method of Claim 29, wherein said endocrine hormone is androgen, testosterone, or androsterone.

61. (New) The method of Claim 29, wherein said endocrine hormone is an adrenal cortex hormone.

62. (New) The method of Claim 29, wherein said endocrine hormone is cortisol, aldosterone, corticosterone or cortisone.

63. (New) The method of Claim 29, wherein said endocrine hormone is an amino acid derivative hormone.

64. (New) The method of Claim 29, wherein said endocrine hormone is triiodothyronine (T3), thyroxine (T4) or a parathyroid hormone.

65. (New) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing a cell that has not been genetically engineered that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or

(C) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or

(D) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression

pattern (4) of said cell; and

(E) comparing gene expression pattern (1) with gene expression pattern (2), (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2), (3) and/or (4) is indicative of endocrine disrupting action by the test substance.

REMARKS

Claims 29-65 will be pending upon entry of this amendment. Independent Claims 29 and 65 track and find support in original Claims 1-4. Support for the limitation of Claim 65 to cells that are not genetically engineered is found in the specification at page 11, lines 6-9--as discussed in the interview, the Applicants submit that this limitation places this claim in condition for allowance.

Dependent Claims 30-34 find support in the original Claims 1-4 and on pages 12-14 of the specification. Methods involving comparison of RNA, cDNA, or protein expression, or by protein modification as covered by Claims 35-47 are described at page 13, lines 14-17, pages 22-31 (polynucleotides), and pages 31-46 (protein and protein modification). The cells recited by Claims 48-56 are described in the specification at page 11. The endocrine hormones recited by Claims 57-64 are described at pages 10 and 11 of the specification. Accordingly, the Applicants do not believe that any new matter has been added.

The Applicants thank Examiner Chakrabarti for the courteous and helpful interview of September 16. It was suggested that limitation of the claims to non-genetically engineered cells would likely address the prior art rejection. Accordingly, Claim 65 is now so limited. New independent Claims 29 and 65 track original Claims 1-4, but have been revised to

further clarify or further distinguish the claimed method from the prior art. These claims are directed to a method of detecting an endocrine disrupting activity of a test substance by comparison of gene expression patterns, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2), (3) and/or (4) is indicative of **endocrine disrupting action** by the test substance. Applicants believe that these amendments address the remaining prior art issues and respectfully request entry and favorable consideration of this amendment and allowance of this application.

Rejection--35 U.S.C. 102(e)

Claims 1-4 and 24 were rejected under 35 U.S.C. 102(e) as being anticipated by Lonial et al., U.S. Patent 6,001,560. This rejection is moot in view of the cancellation of Claims 1-4 and 24.

This rejection would not pertain to new independent Claims 29 and 65, because these claims are directed to a method of detecting the endocrine disrupting action of a test substance by comparing at least two gene expression patterns, where increased or decreased expression of a gene in the gene expression pattern is indicative of endocrine disrupting action by the test substance. Moreover, as discussed with the Examiner, Claim 65 is now limited to non-genetically engineered cells, whereas the method of Lonial involves a genetically engineered cell incorporating a reporter gene.

Lonial is directed to a simple promoter gene assay for determining whether a compound exerts IFN-γ activity and not to a method for determining whether a test substance disrupts the activity of an endocrine hormone present in the medium in which a cell is cultured.

Lonial does not require that the cells used in the described method be sensitive to an endocrine hormone and does not contemplate determining whether the response of the test cells to an endocrine hormone is disrupted by a test substance. Lonial does not suggest comparison of gene expression patterns of cells sensitive to a particular endocrine hormone in the presence and absence of a test substance in order to determine the endocrine disrupting activity of the test substance.

The Applicants submit that the Examiner's concern that the method of Lonial may read on the presently claimed method is misplaced. The Examiner was concerned that the method of Lonial might involve the comparison of gene expression patterns of a cell population exposed to a test substance with a similar cell population not exposed to the test substance. See e.g. Table 1 in col. 10 of Lonial, which compares reporter gene expression (growth hormone) by transformed test cells exposed to, and not exposed to, a substance with IFN-gamma activity (IFN-gamma). While Table 1 of Lonial describes different amounts of reporter gene expression by the transformed cells when exposed or not exposed to IFN-gamma, this method does not determine the endocrine disrupting activity of the test substance (IFN-gamma).

Moreover, while Lonial, col. 11, lines 10-17, mentions a test involving dexamethasone, this test does not involve determining endocrine disrupting effects of the test substance (i.e. IFN-gamma) on the normal cellular effects of dexamethasone. This assay is, in fact, merely a control assay analogous to the control assay using monoclonal antibody B27, which is an IFN-gamma neutralizing antibody (col. 8, lines 22-24). The Lonial dexamethasone test is not concerned with measuring the effects of the test substance (IFN-gamma) on cells grown in the presence of dexamethasone, but is concerned with establishing the utility of the Lonial system for detection of IFN-gamma antagonists, see col. 11, lines 12

and 13. Cols. 11-12 indicate that addition of dexamethasone inhibited expression of the reporter gene (growth hormone) in cells exposed to IFN-gamma compared to cells exposed to IFN-gamma, but not dexamethasone. Such an assay would not (1) determine the endocrine disrupting activity of the test substance or involve comparison of gene expression patterns, wherein the increased or decreased expression of a gene is indicative of the endocrine disrupting activity of the test substance, because the increased expression of the Lonial reporter gene (growth hormone) is indicative of the action of the antagonistic effects of dexamethasone on IFN-gamma, and is not used to determine the endocrine disrupting activity of IFN-gamma on dexamethasone.

Additionally, Lonial is limited to use of transformed cells that express a growth hormone reporter gene and thus would not pertain to Claim 65, which is limited to methods involving cells that are not genetically engineered.

Accordingly, as Lonial does not disclose a method for detecting the endocrine disrupting action of a test substance by comparing gene expression patterns of cells grown in the presence of both the endocrine hormone and the test substance, with cells grown in the absence of the test substance, wherein the differences in the two gene expression patterns are indicative of the endocrine disrupting activity of the test substance, nor disclose methods using cells that are not genetically engineered, the Applicants respectfully submit that this rejection would not apply to Claims 29-65.

Rejection--35 U.S.C. 103

Claims 7, 9, 15-17, 26 and 28 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281. This rejection is moot in view of the cancellation of these claims. As discussed

above, the cited art would not render the claimed invention obvious, because Lonial and Gilles do not suggest a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance as a measure of the endocrine disrupting activity of the test substance.

As discussed above, Lonial is directed to determining whether a substance exhibits an IFN-γ like activity based on expression of a reporter gene (e.g. a growth hormone gene) and is not concerned with whether a test substance (e.g., dioxin) exhibits an endocrine disrupting action.

Gilles is directed to a method for enhancing the production of proteins in eukaryotic cells and does not describe a method for testing whether a test substance (e.g., dioxin) exhibits an endocrine disrupting action. Fig. 2 of Gilles shows an autoradiogram of radiolabelled proteins produced by cell lines transfected with different plasmids; Fig. 7 shows a Southern blot comparing the DNA's of cells transfected with different plasmids; and Fig. 8 shows a Northern blot comparing the RNA's of cells transfected with different plasmids. However, none of these assays were performed by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and putative endocrine disrupting test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance.

Moreover, none of the cited prior art suggests the advantages of the present invention which are shown in the Declaration attached to the previous response. As shown in Table 3 of the Declaration, the present invention makes it possible to detect the difference in the expression numerous different genes associated with an endocrine disrupting action, when

such gene expression was not be detected by a comparable conventional method (e.g., a method that did not culture cells in the presence of an endocrine hormone). Graph 1 of the Declaration shows the effects of dioxin on five different types of genes specifically identified by the method of the present invention in the presence of the endocrine hormone T3. However, conventional methods that did not culture cells in the presence of the T3 hormone, did not identify the ability of dioxin to disrupt these genes or identify the sensitivity of these genes to dioxin.

Accordingly, as the prior art does not disclose or suggest the invention, or the superior ability of the invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption, the Applicants respectfully submit that this rejection would not apply to Claims 29-65.

Rejection--35 U.S.C. 103

Claims 8, 10 and 27 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Pearson et al, U.S. Patent 5,916,779. This rejection is moot in view of the cancellation of these claims.

As discussed above, Lonial does not disclose or suggest the present invention. Pearson does not remedy the deficiencies of Lonial, as neither document suggests a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance as a measure of the endocrine disrupting activity of the test substance.

Pearson is cited for its teachings of RT PCR on RNA recovered from a cell, however, it does not remedy the deficiencies of Lonial, because it does not suggest detecting endocrine disrupting substances by a differential display method in the presence of an endocrine hormone. Moreover, the cited prior art does not suggest the superior sensitivity of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption as shown in the prior Declaration. Accordingly, the Applicants respectfully submit that this ground of rejection would not apply to Claims 29-65.

Rejection--35 U.S.C. 103

Claims 6 and 25 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Comoglio et al., U.S. Patent 6,030,949, further in view of Cubicciotti, U.S. Patent 6,287,765 B1. This rejection is moot in view of the cancellation of these claims.

Lonial in combination with Comoglio and Cubicciotti would not render the invention of Claims 29-65 obvious, as these documents do not suggest a method for detecting an endocrine disrupting action of a test substance by (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance as a measure of the endocrine disrupting activity of the test substance.

Comoglio, Examples 2 and 3, is cited for its teaching of Neuro2a cells and Cubicciotti for its teaching of the hormone triiodothyronine. However, neither of these documents discloses the method of the present invention involving (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the differential gene expression patterns of cells exposed to the test substance and

the cells not exposed to the test substance. Thus, these documents do not remedy the deficiencies in Lonial. Moreover, the cited prior art does not suggest the superior ability of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption as shown in the prior Declaration. Accordingly, the Applicants respectfully submit that this ground of rejection would not apply to Claims 29-65.

Rejection--35 U.S.C. 103

Claims 18 and 19 were rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281, further in view of Comoglio et al., U.S. Patent 6,030,949, further in view of Cubicciotti, U.S. Patent 6,287,765 B1. This rejection is moot in view of the cancellation of these claims. New independent Claims 29 and 65 would not be subject to this rejection for the reasons discussed above.

Rejection--35 U.S.C. 103

Claim 20 was rejected under 35 U.S.C. 103(a) as being unpatentable over Lonial et al., U.S. Patent 6,001,560, in view of Gilles et al., U.S. Patent 4,663,281 further in view of Makari, U.S. Patent 4,752,471. This rejection is moot in view of the cancellation of Claim 20. As discussed above, Lonial and Gilles do not disclose or suggest a method of (1) culturing a cell sensitive to an endocrine hormone in the presence of the endocrine hormone and test substance and (2) comparing the gene expression patterns of cells exposed to the test substance and the cells not exposed to the test substance as a measure of the endocrine disrupting activity of the test substance.

Makari, Claim 5, is cited for its teaching of recovering a glycoprotein by cutting off the polysaccharide chain. However, Makari does not remedy the deficiencies of Lonial or Gilles, as discussed above. Moreover, the cited prior art does not suggest the superior sensitivity of the present invention for identifying particular endocrine disrupting compounds or genes responsive to such disruption as shown in the prior Declaration. Accordingly, this rejection would not apply to Claims 29-65.

CONCLUSION

In view of the above amendments and remarks, the Applicants respectfully submit that this application is now in condition for allowance. Early notification to that effect is earnestly solicited.

Respectfully submitted,

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